



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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<b>DECLARATION UNDER 37 C.F.R. 1.132</b>  Address to: Assistant Commissioner for Patents Washington, D.C. 20231	Attorney Docket No.	STAN-299
	Confirmation No.	1120
	First Named Inventor	NUSSE, ROELAND
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	Group Art Unit	1654
Examiner Name		

DECLARATION UNDER 37 C.F.R. §1.132

Sir:

I, Dr. Karl Willert, do hereby declare as follows:

I am a co-inventor of the above captioned patent application. I have read and understood the Office Action of September 20, 2005, and the references cited therein, particularly with respect to the rejection of claims 7-11 and 13-15 as being unpatentable over Rodan et al., U.S. Patent no. 5,780,291.

The presently claimed invention is directed to methods of isolating biologically active wnt proteins comprising a lipid moiety. Wnt signaling is involved in numerous events in animal development including the proliferation of stem cells and the specification of the neural crest. Wnt proteins are therefore potentially important reagents in expanding specific cell types, but in contrast to other developmental signaling molecules such as the Hedgehogs and the BMPs, prior to the methods of the present invention, Wnt proteins had not been isolated in an active form. Although Wnt proteins are secreted from cells, secretion is usually inefficient and previous attempts to characterize Wnt proteins have been hampered by their high degree of insolubility.

The present application (which findings were subsequently published in the journal *Nature* as Willert *et al.* (2003) *Nature* 423, 448-452) is based on experiments performed by myself and Dr. Nusse on isolation of active Wnt molecules. We found that the Wnt protein is post-translationally modified through the addition of a lipid moiety thereby rendering the protein

highly hydrophobic. Subsequent mass spectrometry confirmed the presence of such a covalently attached lipid on the Wnt polypeptide. Enzymatic removal of the lipid moiety resulted in loss of activity, indicating that the lipid is important for biological activity.

The unexpected finding that wnt proteins comprise a lipid moiety allowed the development of a method for isolating biologically active by performing the isolation in the presence of a detergent sufficient to maintain solubility of the Wnt protein.

The Office Action has rejected the claims of the present application as anticipated by the teachings of Rodan *et al.*, U.S. Patent no. 5,780,291, in particular citing Example 7 of the patent (column 15, lines 1-24). That example reads as follows:

## 15

### EXAMPLE 7

#### Purification of Recombinant Wnt-x

Recombinantly produced Wnt-x may be purified by antibody affinity chromatography.

Wnt-x antibody affinity columns are made by adding the anti-Wnt-x antibodies to Affigel-10 (Biorad), a gel support which is pre-activated with N-hydroxysuccinimide esters such that the antibodies form covalent linkages with the agarose gel bead support. The antibodies are then coupled to the gel via amide bonds with the spacer arm. The remaining activated esters are then quenched with 1M ethanolamine HCl (pH 8). The column is washed with water followed by 0.23M glycine HCl (pH 2.6) to remove any non-conjugated antibody or extraneous protein. The column is then equilibrated in phosphate buffered saline (pH 7.3) together with appropriate membrane solubilizing agents, if necessary such as detergents, and the cell culture supernatants or cell extracts containing Wnt-x or Wnt-x fragments are slowly passed through the column. The column is then washed with phosphate-buffered saline together with detergents, if necessary until the optical density (A280) falls to background, then the protein is eluted with 0.23M glycine-HCl (pH 2.6) together with detergents, if necessary. The purified Wnt-x protein is then dialyzed against phosphate buffered saline.

I have reviewed the text of this Example, and respectfully submit that the method set forth in the Example could not be used to isolate a Wnt protein comprising a lipid moiety to produce a biologically active, substantially homogeneous composition, as set forth in the claims of the present application.

Specifically, the method described for isolation of Wnt-X states that the purified Wnt-X is dialyzed against phosphate buffered saline. Such treatment would cause a wnt protein comprising a lipid moiety to precipitate, and thus biological activity of such a Wnt protein would not be maintained in a substantially homogeneous composition. The reference therefore does not teach a method of isolating a Wnt protein comprising a lipid moiety to produce a biologically active, substantially homogeneous composition.

I hereby declare that all statements made herein of my own knowledge are true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

Date: 1/13/06

By Karl Willert  
Karl Willert, Ph.D.